Genetic Markers and Quantitative Genetic Variation in *Medicago truncatula* (Leguminosae): A Comparative Analysis of Population Structure

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Manuscript received August 29, 1995
Accepted for publication April 24, 1996

ABSTRACT

Two populations of the selfing annual *Medicago truncatula* Gaertn. (Leguminoseae), each subdivided into three subpopulations, were studied for both metric traits (quantitative characters) and genetic markers (random amplified polymorphic DNA and one morphological, single-locus marker). Hierarchical analyses of variance components show that (1) populations are more differentiated for quantitative characters than for marker loci, (2) the contribution of both within and among subpopulations components of variance to overall genetic variance of these characters is reduced as compared to markers, and (3) at the population level, within population structure is slightly but not significantly larger for markers than for quantitative traits. Under the hypothesis that most markers are neutral, such comparisons may be used to make hypotheses about the strength and heterogeneity of natural selection in the face of genetic drift and gene flow. We thus suggest that in these populations, quantitative characters are under strong divergent selection among populations, and that gene flow is restricted among populations and subpopulations.

MOST species cannot be considered as single panmictic units. Spatial variation in morphology and gene frequency is abundant in many species of plants and animals (LEVIN 1988; HAMRICK and GODT 1989; VENABLE and BURQUEZ 1989; ARGYRES and SCHMITT 1991; GEHRING and LINHART 1992; CAPY *et al.* 1993, 1994; MICHALAKIS *et al.* 1993; KALISZ and WARDLE 1994). The heterogeneous distribution of genetic variation within and among populations is provided by mutation, genetic drift due to finite population size, and heterogeneous natural selection, while gene flow and homogeneous directional selection tend to produce genetic homogeneity (EHRLICH and RAVEN 1969; ENDLER 1977; LEVIN 1979; LOVELESS and HAMRICK 1984; SLATKIN 1987). One of the central questions facing population geneticists is the relative degree to which random processes and natural selection lead to genetic differentiation. Neutral loci, when not in linkage disequilibrium with nonneutral loci, provide estimates of the amount of gene flow and genetic drift. Molecular markers, as well as some morphological markers, are usually considered to be selectively neutral (KIMURA 1983), but their adaptive value often remains unknown or questioned (HEYWOOD and LEVIN 1985; NEVO *et al.* 1986, 1991; ALLARD *et al.* 1993; LÖNN 1993; BEGUN and AQUADRO 1994). Life-history characters, in contrast, are usually assumed to undergo natural selection (CHARLESWORTH 1990). Population differentiation observed using quantitative characters is usually considered to be the result of heterogeneous selective pressures. Given that the evolution of single-locus polymorphism and polygenic variation is not necessarily the same (LEWONTIN 1984; LANDE and BARROWCLOUGH 1987), knowledge of patterns of genetic variation using markers and quantitative traits is required before starting any program of gene conservation (HAMRICK *et al.* 1991; SCHAAL *et al.* 1991). The pattern of genetic variation among natural populations of quantitative traits can be compared with that of molecular or other supposedly neutral genetic markers to determine the relative importance of natural selection and migration in the process of differentiation (FELSENSTEIN 1986; ROGERS 1986). Theoretical studies (LANDE 1977; CHAKRABORTY and NEI 1982; ROGERS and HARPENDING 1983; LANDE 1992) have attempted to test population differences in quantitative characters under the expectation of neutral phenotypic evolution. In practice, neutral phenotypic evolution is difficult to estimate in the absence of historical information about rate of mutation, time since divergence and population size. The population structure of quantitative characters may be compared, however, to that of neutral markers to examine whether divergence on both types of characters are due to similar evolutionary processes (PRICE *et al.* 1984; SCHWAEGERLE *et al.* 1986; HAMRICK 1989; LAGERCRANTZ and RYMAN 1990; BACHMANN and VAN HEUSDEN 1992; PROUT and BARKER 1993; SPITZE 1993). Although some authors have suggested that such comparisons are meaningless (LEWONTIN 1984), others (FELSENSTEIN 1986; ROGERS 1986; PROUT and BARKER 1993; SPITZE 1993; PODOLSKY and HOLTSFORD 1995) have shown that instead they are relevant. ROGERS and HARPENDING (1983) showed that one polygenic character should contain exactly as much information about population relationships as one single-locus marker.

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Further theory developed by FELSENSTEIN (1986) and LANDE (1992), and used by SPITZE (1993) and PODOLSKY and HOLTSFORD (1995), also showed that the neutral expectation for a given component of genetic variation in a quantitative trait should not differ from that of a single-locus marker when measured using F_{ST}.

According to PODOLSKY and HOLTSFORD (1995), a comparison between quantitative traits and allozymes for an organism that has high allozyme estimates of Fst, such as a self-pollinating (selfing) plant, remains to be conducted. In this study, we compare the fine-scale spatial structure of 24 quantitative characters and 22 dominant genetic markers [random amplified polymorphic DNA (RAPDs) and one morphological marker] in a species with a selfing rate of ~97.5% (BONNIN *et al.* 1996) and overall F_{st} of the order of 0.40. The reason we used RAPDs and not allozymes is that the level of allozyme polymorphism was very low in the particular populations we studied. We have described elsewhere (BONNIN *et al.* 1996) the genetic structure of the selfing annual plant species *Medicago truncatula* Gaertn. using these dominant markers. Four natural populations (200 km apart one from another) were subdivided into three more or less spatially isolated subpopulations (10–50 m apart). In each subpopulation, one plant was collected every meter alongside a 30 m transect and transplanted into a greenhouse. Offspring of each original plant were obtained from spontaneous selfing and studied with 21 RAPD markers and one morphological marker [pod coiling direction, with anticlockwise *sensu* LESINS and LESINS (1979) monogenic dominant]. In agreement with what one might expect in a selfing species, we found strong differentiation among populations (BONNIN *et al.* 1996). Using a multilocus approach, two populations (populations from Hérault and Corsica) were composed of a few predominant lines. Unexpectedly, the two other populations (populations from Var and Aude) were much more polymorphic and structured. The Aude population had the highest level of polymorphism and differentiation: only one multilocus genotype was found common to two subpopulations (BONNIN *et al.* 1996). In this paper, we compare the differentiation for quantitative traits and for neutral loci between and within the two most polymorphic populations (Aude and Var). Relatively few linkage disequilibria between markers were previously found in Aude and Var populations (BONNIN *et al.* 1996). Thus, we do not expect to find particular agreement between patterns given by the two types of characters.

MATERIALS AND METHODS

Experimental design: Thirty-three genotypes (original plants) from the Var population and 44 from the Aude population were studied. In Var, 12 genotypes came from subpopulation 1, 12 from subpopulation 2, and nine from subpopulation 3. In Aude, 14 genotypes came from subpopulation 1, and 15 genotypes came from each of the subpopulations 2 and 3.

To avoid maternal effects, several seeds per original genotype were obtained by two or three generations of spontaneous selfing in the greenhouse. They were scarified and sown in Petri dishes on wet filter paper before being vernalized at 10° for 8 days. After germination to take into account environmental variance within genotype (FALCONER 1981), five seeds per genotype were individually planted in small pots, giving a total of 385 plants. The pots were placed in a randomized block design on greenhouse benches. During seedling growth, cotyledon emergence date and sixth leaf emergence date were scored. Length and breadth of the first leaf were also measured (at the six leaves stage) and area of the first leaf was computed from these measurements. After 40 days, the plants were transplanted into larger pots (with the same pattern of randomized blocks) in a covered garden. The growth characters measured were the length of the main stem and the length of secondary stems at two different dates and at the flower bud stage. From these data, the daily growth of the main and secondary stems was computed. The dates of first flower bud, first open flower, first unripe pod, and first ripe pod were also scored. The reproductive interval between the age at which the first flower opened and the age at which the first pod matured was calculated. Finally, at ripeness, the above ground biomass was separated into vegetative material (stems) and reproductive material (pods). For weight determination, the material was dried at 60 for 8 days. The weight of the stems, the pods as well as the total number of pods were determined. From these measurements, the total weight of each plant, the weight of 100 pods, and the reproductive effort were computed. The 24 quantitative traits are listed in Table 1. All phenological traits are given in days after planting in the greenhouse.

Data analysis: The organization of genetic variation in *M. truncatula* was revealed by partitioning the variance in gene frequencies and in mean value of quantitative characters among hierarchical levels of genetic organization. Differences in population, subpopulation and genotype means were examined for each metric character using a nested analysis of variance for unequal sample sizes using type III SS of SAS (Proc GLM; SAS Institute, Inc., 1985). The model included population, subpopulation within population, genotype within subpopulation within population, block. To estimate variance components, population, subpopulation and genotype were considered as random effects, whereas block was fixed. To test the significance of differences in gene frequency between and within populations, analysis of variance using SAS Proc GLM (type III) was also performed for each neutral marker on allelic frequency per individual including the effects: population, subpopulation within population (WEIR 1990).

Partitioning of the variance among hierarchical levels allowed us to estimate components of genetic variance for both types of characters using coefficients of expected mean squares.

Variance components arising between populations (σ^2_P), between subpopulations within populations (σ^2_{SP}), between genotypes within subpopulations within populations (σ^2_G), and due to error (arising between individuals within original genotype) (σ^2_e) were calculated for each quantitative trait. Environmental variance (σ^2_e) was taken away from total phenotypic variance ($\sigma^2_{TP} = \sigma^2_e + \sigma^2_G + \sigma^2_{SP} + \sigma^2_P$) to determine portions of total genetic variance ($\sigma^2_{TG} = \sigma^2_G + \sigma^2_{SP} + \sigma^2_P$) arising between populations (σ^2_P/σ^2_{TG}), between subpopulations within populations ($\sigma^2_{SP}/\sigma^2_{TG}$), and between genotypes within subpopulation within population (σ^2_G/σ^2_{TG}). This method was that used by VENABLE and BURQUEZ (1989). The quantity σ^2_P/σ^2_{TG} is equivalent to F_{st} for a quantitative trait in a completely selfing species. We give in APPENDIX the expected F_{st}

TABLE 1

List of characters measured in this investigation and their abbreviations

Character	Abbreviation
Seedling traits	
1. Emergence date of cotyledons	DCOT
2. Emergence date of sixth leaf	D6F
3. Length of the first leaf	LGFT6
4. Breadth of the first leaf	LRFT6
5. Area of the first leaf	SFT6 = LGFT6*LRFT6
Growth traits	
6. Length of the main stem 62 days after planting	LG1J62
7. Length of the main stem 96 days after planting	LG1J96
8. Length of secondary stems 62 days after planting	LGMJ62
9. Length of secondary stems 96 days after planting	LGMJ96
10. Daily growth of the main stem	LG134 = (LG1J96-LG1J62)/34
11. Daily growth of secondary stems	LGM34 = (LGMJ96-LGMJ62)/34
12. Length of the main stem at the flower bud stage	LG1JBF
13. Length of secondary stems at the flower bud stage	LGMJBF
Reproductive traits	
14. Date of first flower bud	DBF
15. Date of first open flower	DFO
16. Date of first unripe pod	DGV
17. Date of first ripe pod	DGB
18. Reproductive interval	GBFO = DGB-DFO
19. Weight of dried stems	PTIG
20. Weight of dried pods	PGOU
21. Number of pods	NGOU
22. Total weight	PTOT = PTIG + PGOU
23. Weight of 100 pods	P100G = 100*PGOU/NGOU
24. Reproductive effort	REPRO = PGOU/PTOT

Leaves and branchs lengths were measured in centimeters, weights in milligrams.

of a quantitative character for a species that shows any intermediate level of inbreeding. In our study, we have assumed complete selfing. If some outcrossing occurs from time to time, the environmental component of variance estimates include some genetic variance as well. As a consequence, and providing these outcrossing events occur at the subpopulation level only, σ^2_G might be slightly underestimated. It is unlikely however that the results will be very much affected, given the large selfing rate usually observed in *M. truncatula*.

To take into account the fact that some characters had less genetic variability, we also present variance components as portions of total genetic variance, weighed by the heritability of the corresponding trait ($H^2 = \sigma^2_{TG}/\sigma^2_{TP}$), which amounts to study portions of total phenotypic variance (σ^2_P/σ^2_{TP}, $\sigma^2_{SP}/\sigma^2_{TP}$, σ^2_G/σ^2_{TP}). This is the method that was used by SCHWAERGERLE *et al.* (1986). Because this method gives a larger weight to genetically more variable traits, it might bias the result toward given kinds of quantitative traits (*i.e.*, those whose polymorphism is maintained by balancing selection or conversely those that are more neutral). It is thus not clear which method is best.

Genetic variance for each assumed neutral locus was also partitioned into variance components [σ^2_P for the between populations component, σ^2_{SP} for the between subpopulations within population component, σ^2_G (the "error" component) due to the between genotypes within subpopulations within populations component, and $\sigma^2_{TG} = \sigma^2_G + \sigma^2_{SP} + \sigma^2_P$ for the total genetic variance]. Differentiation between populations was measured by the portion of total genetic variance arising between populations ($F_{st} = \sigma^2_P/\sigma^2_{TG}$). The portion of variance in gene frequencies due to differentiation among subpopulations within populations was estimated by $\sigma^2_{SP}/\sigma^2_{TG}$. The quantity σ^2_G/σ^2_{TG} represents differentiation among genotypes within subpopulations, within populations.

As for quantitative characters, there are two ways of transforming variance components, depending on whether the proportion of variance at an individual locus explained by a given component is weighed by the overall genetic variability at that locus, or whether proportions of total variance are first calculated for each locus. WEIR and COCKERHAM (1984) suggested that the first estimates were unbiased. As for quantitative traits, this method gives a larger weight to more variable loci. SCHWAERGERLE *et al.* (1986), however, used the second method. In this paper, both methods are presented.

For each quantitative character and each marker, we also calculated $\sigma^2_{SP}/(\sigma^2_{SP} + \sigma^2_G)$, the proportion of within population genetic variance explained by population structure. This ratio was then averaged over quantitative characters and over markers.

Sampling variance on estimates of variance components can be obtained using bootstrap or Jackknife procedures. As suggested by WEIR (1990), we used bootstrapping over loci (5000 bootstraps) to calculate means and 95% confidence intervals from the observed distribution. Bootstrapping over a set of traits implicitly assumes that these traits are independent (VAN DONGEN 1995). Whereas few linkage disequilibria were observed among loci (BONNIN *et al.* 1996), some characters were correlated. Thus, although we also produce confidence intervals by bootstrapping over quantitative characters, those should be treated with caution. An alternative method is bootstrapping over individuals and families (PROUT and BARKER, 1993; SPITZE 1993; LONG and SINGH, 1995). As shown

by VAN DONGEN and BACKELJAU (1995) the power of this procedure seems to decrease with small sample sizes. To use this resampling method is like producing a new generation out of the sample itself, so that confidence intervals obtained this way are in fact not those of the parameters of the population under study (J. GOUDET, personal communication). At the subpopulation level, our sample sizes were <20. However, since the bootstrapping method is commonly used, and because it was the only way to obtain confidence intervals per character, we also give 95% confidence intervals of overall statistics per character obtained this way. For each character, 1000 bootstraps were generated.

Given that correlations between quantitative traits and linkage disequilibria between genetic markers can inflate the values of the overall statistics calculated, we also performed multivariate analyses for both types of characters to obtain linear combinations of the original variables and to estimate genetic variance components using these new independent variables. A principal component analysis (proc PRINCOMP, SAS Institute, Inc., 1985) and a correspondence analysis (a weighted principal component analysis of a contingency table, proc CORRESP, SAS Institute, Inc., 1985) were computed for quantitative traits and genetic markers, respectively. The coordinates of the individuals on the axes defined in the multivariate analyses were used to calculate genetic variance components. The statistics previously described were estimated for each new variable, weighting each value by the portion of variance explained by each axis in the multivariate analysis.

RESULTS

Quantitative characters: Table 2 shows the absolute values of the variance components and the partitioning of the variance for metric traits when weighed by trait heritability, that is, when calculated by dividing each component by overall phenotypic variance. A large part of the total phenotypic variance (45%) was found to occur between populations. When the analysis was performed on principal components (PC), the value was similar (41.7%). Genetic variance within populations accounted for only 19.9% (21.7% on PC) of the total phenotypic variance. Differentiation among subpopulations of the same population was very low (2.1% of total phenotypic variance) (2.1% for PC), the remaining 17.8% corresponding to the contribution of genetic variance within subpopulations (19.7% for PC). Table 2 also shows the partitioning of the genetic variance for quantitative characters when not weighed by trait heritability, that is, when calculated by dividing each component by the overall genetic variance. This amounts to calculate F statistics on quantitative characters (see APPENDIX). Again, a large portion (58%, 48% for PC) of the variance is found to occur between populations, and a small part (4.3%, 5.8% for PC) of the genetic variance occurs among subpopulations. Within subpopulation genetic variability explains 37.3% (46.2% for PC) of overall genetic variance. Thus both methods, with or without considering correlations among characters (original data or principal components), indicate a strong differentiation between populations and a low differentiation among subpopulations.

At the population level, only 11.9% (95% CI = 6.5–17.2%) was due to within subpopulation structure in the original sample (average of $\sigma^2_{SP}/(\sigma^2_{SP} + \sigma^2_G)$ over traits).

There was considerable diversity in the partitioning of variance among different characters (Figure 1). Local population structure was low for all characters: <20% of variance was found among subpopulations for any trait (Figure 1). Out of 24 characters, 18 were strongly differentiated between the two populations (out of which 14 had a lower limit of the 95% confidence interval for σ^2_P/σ^2_{TG} (above 58%), whereas for six characters, the between populations component of genetic variance represented <11% (among which five had an upper limit of the 95% confidence interval for σ^2_P/σ^2_{TG} below 43%). Among these six characters, four (LRFT6, LFGT6, SFT6, LGMJ62) had large genetic correlations among them within each population (data not shown), so that only three groups of characters showed little differentiation among populations. These three groups included characters of seedling traits and reproductive interval (GBFO, see Table 1). Among the 18 characters that displayed a strong differentiation between populations, 12 groups of characters could be defined, with one to four genetically correlated characters per group (data not shown). These groups included phenological as well as biomass characters; most of the differentiation between populations was explained by variation in reproductive traits (phenological variables of flowering and traits related to pods production) and variation in growth characters (*e.g.*, daily growth of the main and secondary stems) (Table 2).

Markers: Table 3 shows the absolute values of the variance components for markers, which amounts to weighting the individual locus contribution by its total variance. Variance among populations accounted for only 30.1% of the total variance, whereas a much larger part of the variance (69.9%) was found to occur within populations. The values calculated from the axes of the correspondence analysis (CA) were similar (respectively, 26.5 and 73.5% of total variance). Within populations, among subpopulations variance accounted for 14.9% (16.5% for CA) of the total variance, a figure quite larger than the one observed for quantitative characters (2.1%, see Table 3). The largest contribution came from within subpopulations variance, which accounted for 55% (57% for CA) of the total variance.

Table 3 also shows the partitioning of the genetic variance for markers when calculated by dividing each component by overall genetic variance. Again, a large portion of the variance is found to occur within subpopulations (74%, 70% for CA) and a small part of the genetic variance occurs between populations (13.5%, 15.9% for CA). Between subpopulations genetic variability explains 12.5% (14.1% for CA) of the overall variance. Thus all methods indicate that most variability of genetic markers occurs at the level of the subpopula-

TABLE 2

Partitioning of genetic variation in *M. truncatula* using 24 quantitative characters

Trait	σ^2_ϵ	σ^2_G	σ^2_{SP}	σ^2_P	$\sigma^2_\epsilon/\sigma^2_{TP}$	σ^2_G/σ^2_{TP}	$\sigma^2_{SP}/\sigma^2_{TP}$	σ^2_P/σ^2_{TP}	σ^2_G/σ^2_{TG}	$\sigma^2_{SP}/\sigma^2_{TG}$	σ^2_P/σ^2_{TG}
DCOT	0.7	0.38	0	0.18	0.56	0.30	0	0.14	67.7	0	32.3
D6F	4.22	2.97	0.46	0.12	0.54	0.38	0.06	0.02	83.7	12.9	3.4
LGFT6	0.02	0.01	0	0.00	0.69	0.28	0	0.03	90.3	0	9.7
LRFT6	0.02	0.01	0	0.00	0.54	0.44	0	0.02	96.3	0	3.7
SFT6	0.03	0.02	0	0.00	0.57	0.38	0	0.05	89.2	0	10.8
LG1J62	0.25	0.11	0.06	0.44	0.29	0.13	0.07	0.51	17.9	10.3	71.8
LG1J96	8.56	6.70	1.65	29.94	0.18	0.14	0.04	0.64	17.5	4.3	78.2
LGMJ62	8.43	1.50	0.33	0.15	0.81	0.14	0.03	0.02	76	16.6	7.4
LGMJ96	71.17	62.10	17.67	278.13	0.17	0.14	0.04	0.65	17.4	4.9	77.7
LG134	0.01	0.00	0.00	0.02	0.19	0.15	0.03	0.63	18.2	3.5	78.3
LGM34	0.05	0.05	0.01	0.23	0.14	0.14	0.04	0.68	16.4	4.3	79.3
LG1JBF	5.50	4.18	1.35	14.21	0.22	0.17	0.05	0.56	21.2	6.8	72
LGMJBF	96.08	87.55	21.62	88.61	0.33	0.30	0.07	0.30	44.3	10.9	44.8
DBF	24.58	22.32	0	191.24	0.10	0.10	0	0.80	10.5	0	89.5
DFO	24.06	21.20	0	211.28	0.10	0.08	0	0.82	9.1	0	90.9
DGV	23.75	19.59	0	204.29	0.10	0.08	0	0.82	8.7	0	91.3
DGB	14.45	16.13	1.31	195.83	0.06	0.07	0.01	0.86	7.6	0.6	91.8
GBFO	5.89	1.55	0.05	0.20	0.77	0.20	0.01	0.02	86.2	3	10.8
PTIG	49.07	7.75	4.78	79.80	0.35	0.06	0.03	0.56	8.4	5.2	86.4
PGOU	56.39	22.30	0	150.81	0.24	0.10	0	0.66	12.9	0	87.1
NGOU	10137.13	6406.10	0	6922.27	0.43	0.27	0	0.30	48.1	0	51.9
PTOT	158.93	4.44	3.12	8.68	0.91	0.02	0.02	0.05	27.3	19.2	53.5
P100G	0.72	0.50	0	6.36	0.09	0.07	0	0.84	7.2	0	92.8
REPRO	0.00	0.01	0	0.06	0.06	0.13	0	0.81	14.2	0	85.8
Mean					35.1%	17.8%	2.1%	45%	37.3%	4.3%	58.4%
95% CI						14–22%	1–3%	32–58%	24.6–50.4%	2.3–6.6%	45.1–71.6%
Within population						19.9% (15–25%)			41.6% (28.4–54.9%)		

Variance components at genotype (σ^2_G), subpopulation (σ^2_{SP}), and population (σ^2_P) levels are presented in absolute values. σ^2_ϵ is the environmental variance within family (error term). The components are also expressed as proportions of total phenotypic variance ($\sigma^2_{TP} = \sigma^2_\epsilon + \sigma^2_G + \sigma^2_{SP} + \sigma^2_P$) and as percentages of total genetic variance ($\sigma^2_{TG} = \sigma^2_G + \sigma^2_{SP} + \sigma^2_P$). Confidence intervals (CI) of the overall statistics were obtained computing bootstrap over characters.

tion, and that the amount of differentiation is almost as large among subpopulations as among populations.

At the population level, the contribution of the between subpopulations level was 13.9% (95% CI = 7.3–21.6%), slightly but not significantly larger than the value of 11.9% (6.5–17.2%) found for quantitative characters.

For most markers (18 out of 22 markers) a large part of the variation was due to variance within subpopulations (between 50% and 100% of the total variance) (Figure 2). For each of these 18 markers, the upper limit of the 95% confidence interval for σ^2_P/σ^2_{TG} was below 44%. Two markers out of 22 revealed a larger differentiation between populations (>80% of total variance) (Table 3). For these two markers, the lower limit of the 95% confidence interval for σ^2_P/σ^2_{TG} was above 69%. For one locus (B18-8), the contribution of the among subpopulations component was above 60%. This locus showed no differentiation at all among populations.

DISCUSSION

In the following discussion, we assume that each character is at equilibrium among the various evolutionary forces. Different evolutionary hypotheses may be considered according to the results of comparisons of differentiation among spatial units for different types of characters, *i.e.*, genetic markers *vs.* quantitative characters. We assume that most (if not all) genetic markers are neutral. Those few genetic markers that behavior differs from the majority of genetic markers are likely to undergo some sort of selection (*e.g.*, hitchhiking). The hypothesis being tested is that quantitative characters are not neutral. This hypothesis can be tested only when population structure differs between quantitative and genetic characters. Information about the relative importance of migration and selection can then be inferred.

When no population differentiation among spatial units (populations or subpopulations) can be shown for either type of character, no inference can be made as to whether selection is acting or not on any given character. The only conclusion that can be drawn is that gene flow is not likely to be restricted. Notice however that one could observe no structure even in the absence of actual gene flow, if the spatial units have a common origin and local drift is small; such low local drift would be achieved if for instance each unit was itself subdivided in very small units, such that within

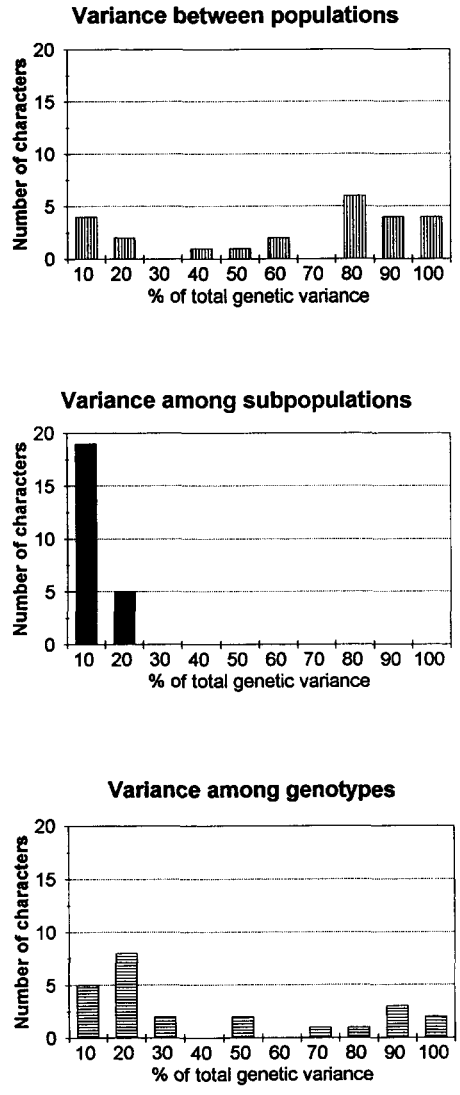

FIGURE 1.—Quantitative characters: frequency histograms of the number of traits as a function of the proportion of total genetic variance explained by each variance component.

subunit drift would be maximal: in average, at the upper level there would be no drift at all. We will ignore such "sub-subdivision" hypothesis in this paper.

When spatial units are differentiated as much for quantitative characters as for genetic (supposedly neutral) markers, gene flow is restricted, and genetic drift (due to finite population sizes) could be the cause of differentiation for both types of characters.

When spatial units are strongly differentiated with respect to quantitative characters, but not to genetic (neutral) markers, it is likely that selection must be strong enough to overcome gene flow and is heterogeneous among spatial units (otherwise there would be no differentiation).

When conversely, spatial units are structured with respect to genetic (neutral) markers, but not to quantitative characters, it is likely that genetic drift is acting on markers, and that selection is homogeneous on quantitative characters (no structure) and strong enough to overcome genetic drift. It should be noticed, however, that homogeneous directional or stabilizing selection in two isolated units could actually increase divergence as compared to drift alone, if initial frequencies of favored alleles are very low (COHAN 1984). This result however is likely to hold only if mutation is limiting (LYNCH 1986) and no gene flow occurs among the two populations. We will thus ignore this prediction in the remaining part of this paper.

Some studies have compared the spatial structure of various types of molecular markers. For instance, KARL and AVISE (1992) have observed that enzyme polymorphism in oysters was less structured than mt-DNA polymorphism. In the same way, POGSON *et al.* (1995) found that enzyme polymorphism in Atlantic cod was much less structured than nuclear restriction fragment length polymorphism (RFLP). In both studies, the authors took their observations as evidence that enzyme polymorphism was undergoing directional homogeneous selection. From above, it is clear that an alternative explanation could be that enzyme polymorphism was neutral, whereas mt-DNA and RFLP polymorphisms were undergoing heterogeneous selection. Conversely, in *Clarkia dudleyana,* PODOLSKY and HOLTSFORD (1995) compared the F_{st} estimates among 11 populations for eight allozyme markers with those for nine discrete and nine continuous morphological traits. They found that the allozymes and seven continuous traits had the lowest F_{st} estimates. Estimates of F_{st} for four discrete and two continuous traits were higher. Whereas the pattern of differentiation for the discrete morphological traits reflected the geographical distribution of populations, the patterns for the other traits did not. The authors suggested that selection had been occurring on the discrete morphological traits, with optima differing among geographical groups. Notice that the reverse conclusion might have been reached, namely that the structure of discrete morphological traits resulted from differentiation due to isolation by distance, whereas allozymes and continuous traits were experiencing homogeneous selection. However, because it is unlikely that all enzyme loci in PODOLSKY and HOLTSFORD' study were undergoing natural selection, the conclusion they reached was probably the most appropriate.

Thus, in the absence of any *a priori* evidence on the neutrality of each type of character, it is difficult to come to a conclusion. Ideally, such comparisons should be performed on traits whose selective status is well-known. For instance, CHEVILLON *et al.* (1995) have shown a low differentiation among populations for allozyme loci in the mosquito *Culex pipiens,* while a strong differentiation was found in the distribution of insecticide resistance genes. This differentiation was closely linked to known insecticide treatments.

In the following discussion, we will assume *a priori* that quantitative traits are more likely to be undergoing natural selection than the molecular and morphologi-

TABLE 3

Genetic variation within and between populations of *M. truncatula* using 22 genetic markers

Locus	σ^2_G	σ^2_{SP}	σ^2_P	σ^2_G/σ^2_{TG}	$\sigma^2_{SP}/\sigma^2_{TG}$	σ^2_P/σ^2_{TG}
B6-550	0.03	0	0.00	98.7	0	1.3
B6-600	0.16	0.06	0.03	67	22.9	10.1
B6-700	0.09	0.05	0	66	34	0
B6-750	0.13	0.06	0.05	53.8	24.2	22
B4-344	0.02	0.00	0	94.1	5.9	0
B4-550	0.01	0	0	100	0	0
B4-800	0.15	0.02	0	87	13	0
B4-850	0.03	0	0.00	98.7	0	1.3
B4-1650	0.05	0	0.41	10.9	0	89.1
B7-480	0.19	0.05	0	77.7	22.3	0
B7-550	0.06	0.01	0.00	85.3	11.8	2.9
B18-480	0.21	0.02	0.06	72.4	7.4	20.2
B18-550	0.01	0	0	100	0	0
B18-600	0.13	0.11	0.06	43.8	35	21.2
B18-800	0.05	0.08	0	36.3	63.7	0
B18-960	0.08	0.01	0.01	84.9	5.6	9.5
B18-1170	0.16	0.00	0.01	93.3	1.9	4.8
B15-500	0.05	0	0.00	94.3	0	5.7
B15-600	0.01	0	0	100	0	0
B15-800	0.11	0.04	0.05	53.4	20.9	25.7
B15-960	0.07	0.00	0.35	16.3	0.4	83.3
Pod coiling	0.10	0.01	0	93.8	6.2	0
Total or mean	1.89	0.51	1.04	74%	12.5%	13.5%
	(55%)	(14.9%)	(30.1%)			
95% CI	40–74%	8–24%	8–49%	62.8–84.4%	6.5–19.9%	4.8–24.5%
Within population		69.9%			86.5%	
		CI = 50–92%			CI = 74.9–95.3%	

Variance components at genotype (σ^2_G), subpopulation (σ^2_{SP}), and population (σ^2_P) levels are presented in absolute values. Components are also expressed as percentages of total genetic variance ($\sigma^2_{TG} = \sigma^2_G + \sigma^2_{SP} + \sigma^2_P$). Confidence intervals (CI) of the overall statistics were obtained computing bootstrap over loci.

cal characters we used. The possibility that the molecular markers we used are not neutral is, however, left open. Overall, the two populations studied are significantly more differentiated for quantitative characters than for markers: 58.4% of total genetic variance was explained by between-population variance for quantitative traits (Table 2) *vs.* 30% or 13.5% for markers depending on which method of averaging was used (Table 3). Given that 20 out of 22 markers displayed little between-population differentiation, compared to within population variability, it is reasonable to assume that neutral genes are characterized by a small contribution of the between-population component of variance. If population differentiation were due only to drift affecting all loci, markers and characters should show the same pattern of variance contributions. Overall, our results thus indicate stronger heterogeneous selection on life-history traits than on markers (Figure 3).

Recently, E. CHAULET (unpublished data) studied 50 North African populations of *Medicago truncatula*. Her experimental design was very different, as she did not measure quantitative traits at the individual but at the plot level (four replicate plots per population). Also, she studied enzyme polymorphism using five polymorphic loci. To compare our data with these results, we reanalyzed our own data assuming the same design (no individual data, no hierarchical sampling design). We found that F_{st} of markers in Algerian populations was 0.31, compared to 0.35 using our data set, and F_{st} on quantitative characters ($\sigma^2_P/(\sigma^2_P + \sigma^2_e)$) was 0.61, compared to 0.46 using our data set. The trends are thus similar. The even larger differentiation among Algerian populations for quantitative characters is probably due to the much larger geographic area that was represented, and thus the larger heterogeneity among habitats encountered.

In response to local selection, each population of the present study displays a homogeneous combination of main traits (individuals from the Aude population grow slower, flower later and produce fewer pods than individuals from Var). It is however difficult to distinguish ecological factors being at the origin of such divergence. Both populations studied occur in open habitats that can be easily disturbed in space and time (population from Aude is bordering a vineyard, while population from Var is on a roadside), and nothing is known about their history. Diversifying selection is probably responsible for the interpopulational divergence in

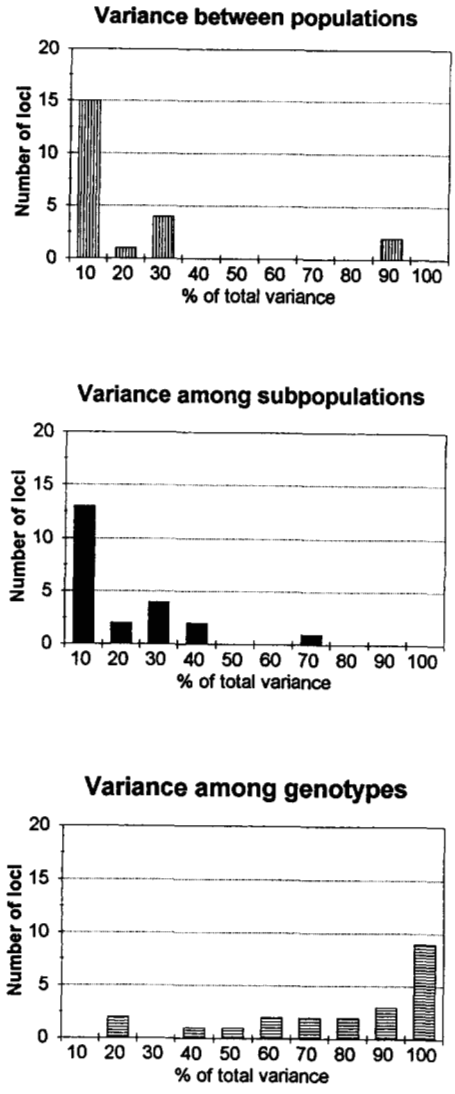

FIGURE 2.—Genetic markers: frequency histograms of the number of traits as a function of the proportion of total genetic variance explained by each variance component.

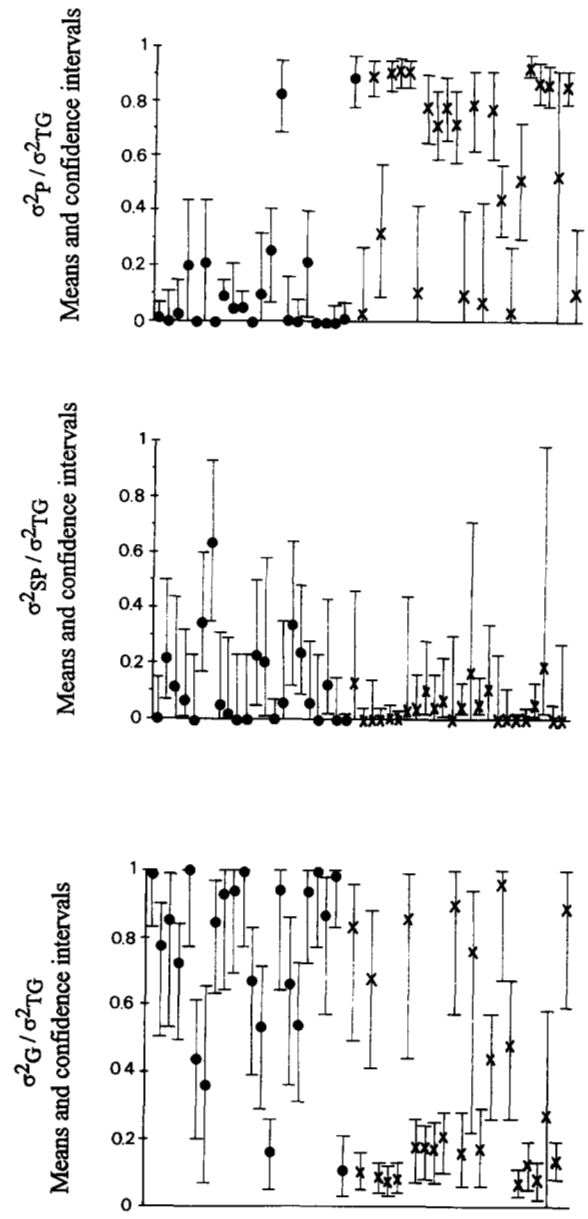

FIGURE 3.—Comparison of variance components between all traits [quantitative characters (×) and markers (●)] studied.

adaptive characters such as flowering date or pod production (related to fitness). Some of the markers may also be subject to selective differentiation by direct effects or hitchhiking. In particular, loci B15-960 and B4-1650 are strongly differentiated among populations, differently from the other loci (Table 3). Conversely, six characters out of 24 showed little differentiation between populations (Figure 3): these were four out five juvenile characters (D6F and the three genetically correlated characters LGFT6, LRFT6 and SFT6, see Table 2), the length of secondary stems 62 days after planting (LGMJ62, also strongly correlated to the previous group), and the time lag between the first flower and the first mature pod (GBFO). Either these characters are neutral, or they are under homogeneous selection. Given that their structure does not significantly differ from markers (Figure 3), the hypothesis that they are neutral cannot be rejected.

Within population, subpopulations were slightly but not significantly more differentiated for markers than for characters: for markers, on average 13.9% of total genetic variability within a population was explained by spatial structure among subpopulations *vs.* 11.7% for quantitative characters. The sign of the small difference is in agreement with what would be expected under the hypothesis that most quantitative characters undergo homogeneous selection among subpopulations. The fact that the difference is not significant, however, suggests that restricted gene flow among subpopulations and local drift are probably the main factors determining genetic differentiation among spatial units at the population level for the quantitative characters that were measured. Alternatively, it could be that selection is heterogeneous among subpopulations. Hitchhiking

would then be responsible for the differentiation of genetic markers. Such correlations between genetic markers and fine-scale environmental variation were for instance found in *Avena barbata* (*e.g.*, ALLARD 1989).

Our previous study of population structure in *Medicago truncatula*, using the same markers and four populations, has also revealed (using a hierarchical design) a larger variance component within (55%) than among (45%) populations (BONNIN *et al.* 1996; the value obtained with a nonhierarchical design was 51%, which is very close to the value usually observed for selfing species, HAMRICK and GODT 1989). The method applied was the one suggested by WEIR and COCKERHAM (1984). The within subpopulations variation contributed to 66.5% of the variance within populations. In the present study, the choice of the most polymorphic populations has led to increase the part of variance arising within populations (69.9%) and the contribution of the within subpopulations variation to this variance (78.7%) (Table 3). The large within subpopulations variance of molecular markers in these populations can be explained by either small neighborhood sizes within subpopulations, by outcrossing following migration events, or both (BONNIN *et al.* 1996). Such an explanation might apply to variation of quantitative traits as well.

Although the largest part of the genetic variance in life-history traits occurred among populations, there was a significant genetic variance within each subpopulation for most traits (Table 2, compare values of σ^2_G and σ^2_e): broad-sense heritabilities within a subpopulation varied from 0.02 to 0.68, depending on the character. This contrasts with results of KALISZ and WARDLE (1994) on *Campanula americana*, who found very little genetic variation at the population level, whereas genetic differentiation among populations was very large for most life-history characters studied. Notice however that the assumption made by all these studies is that the genetic variance measured in controlled conditions reflects that in the field. This is probably not true for most cases, so that inferences concerning natural populations would be difficult to draw.

Other studies have compared quantitative and biochemical variation within species. In *Daphnia obtusa*, SPITZE (1993) found that differentiation among populations for quantitative characters such as body size was significantly larger than the neutral expectation estimated from allozyme polymorphism, while it was smaller for relative fitness. In a comparative study by RITLAND and JAIN (1984), populations of *Limnanthes alba* showed significant among-population genetic variation of certain life history characters, which was correlated with the geographical distribution of populations. In contrast, allozyme variation showed little pattern of differentiation. Similarly, LONG and SINGH (1995) have shown, using pairwise comparisons among populations of *Drosophila melanogaster* along a cline, that two populations can experience considerable gene flow between them (as measured by allozymes) and still be different for a quantitative character. As in our study, not all characters were responding in the same manner to diversifying natural selection.

More congruence between population differentiation for gene frequencies and selected loci is usually observed or expected in selfing than in outcrossing species (PRICE *et al.* 1984; NEVO *et al.* 1986; BACHMANN and VAN HEUSDEN, 1992; LÖNN 1993; but see KHALER *et al.* 1980; BEER *et al.* 1993). Such observations are explained by small recombination rates and slow decay of linkage disequilibria, even for unlinked loci (ALLARD, 1989). In some studies of selfing species, however, *e.g.*, GILES (1984) on *Hordeum murinum*, a very low amount of enzyme polymorphism was found both within and among populations, whereas significant genetic variation was observed for quantitative characters at both levels. Thus, as in our study, little agreement was found between differentiation of genetic markers and life-history traits in a selfing species.

In selfing species, there is a much greater opportunity for correlated response to selection than in outcrossing species. Thus, we expect to find a stronger differentiation among populations experiencing heterogeneous directional selection than in outcrossing species. Although, as in outcrossing species, neutral and selected characters might not have the same spatial structure, they both should be larger in selfing species than in outcrossing species. Not enough published material on spatial structure of quantitative diversity is available to date to test this prediction.

Thanks to YANNIS MICHALAKIS, MARK KIRKPATRICK and FRANÇOIS ROUSSET for stimulating discussions. MICHEL RAYMOND and JOËLLE RONFORT read a preliminary version of this paper. Thanks to JÉRÔME GOUDET for pointing out to us problems associated to the use of bootstraps over individuals. MONTY SLATKIN and YANNIS MICHALAKIS made useful comments on the last draft. Two anonymous reviewers made helpful criticisms. ANNE-MARIE DUFFOUR provided a great technical help with the literature survey; BENJAMIN JOUVE and NADÈGE COURTES were very helpful with the greenhouse experiments. I.B. acknowledges a grant from the Ministry of Research and Technology. Financial support for the experiments was provided by the Institut National de la Recherche Agronomique and the European Economic Community. This is publication Number ISEM95-092 of the Institut des Sciences de l'Evolution, Université Montpellier 2.

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Communicating editor: B. S. WEIR

APPENDIX: F_{st} OF A QUANTITATIVE TRAIT UNDER LOCAL INBREEDING

This appendix is a follow-up of the treatment by WRIGHT (1969, pp. 446–448). It will be assumed that the effects of loci and environment on a quantitatively varying character are additive. We assume furthermore that allelic effects at each locus are also additive.

WRIGHT has shown that in a subdivided population, the total variance of a quantitative trait is equal to

$$\sigma^2_{IT(F)} = (1 + F_{IT(0)})^2, \tag{1}$$

where $F_{IT(0)}$ is the inbreeding coefficient at the whole population level.

WRIGHT then calculated the average variance within subpopulation, $\bar{\sigma}^2_{IS}$, for any given within-subpopulation inbreeding coefficient (F_{IS}), and the component of total variance attributable to variance of the character mean among subpopulations, σ^2_{ST}, with the corresponding inbreeding coefficient being F_{ST}.

If each subpopulation is panmicitic ($F_{IS} = 0$), F_{ST} does not change, whereas $F_{IT} = F_{ST}$. The variance of individuals within total is then

$$\sigma^2_{IT} | (F_{IS} = 0) = (1 + F_{ST})\sigma^2_{IT(0)}. \tag{2}$$

The expected variance within each subpopulation if panmictic is

$$\bar{\sigma}^2_{IS} | (F_{IS} = 0) = (1 - F_{ST})\sigma^2_{IT(0)}. \tag{3}$$

Since the total variance is the sum of the components, σ^2_{ST} is the difference between (2) and (3):

$$\begin{aligned}\sigma^2_{ST} &= (1 + F_{ST})\sigma^2_{IT(0)} - (1 - F_{ST})\sigma^2_{IT(0)} \\ &= 2F_{ST}\sigma^2_{IT(0)}.\end{aligned} \tag{4}$$

Thus one can write

$$F_{ST} = \frac{\sigma^2_{ST}}{\sigma^2_{IT(0)}}. \tag{5}$$

The average variance within each subpopulation if inbred is (WRIGHT 1969, p. 448)

$$\begin{aligned}\bar{\sigma}^2_{IS} &= \sigma^2_{IT} - \sigma^2_{ST} \\ &= (1 + F_{IT} - 2F_{ST})\sigma^2_{IT(0)}.\end{aligned} \tag{6}$$

From what preceedes, it follows that

$$\begin{aligned}\sigma^2_{IT(0)} &= \frac{\bar{\sigma}^2_{IS}}{1 + F_{IT} - 2F_{ST}} \\ &= \frac{\bar{\sigma}^2_{IS}}{(1 + F_{IS})(1 - F_{ST})}.\end{aligned} \tag{7}$$

By replacing, in the expression (5) of F_{ST}, $\sigma_{IT(0)}$ by the expression above, one obtains

$$F_{ST} = \frac{\sigma^2_{ST}(1 - F_{IS})(1 - F_{ST})}{2\bar{\sigma}^2_{IS}}, \tag{8}$$

and thus

$$F_{ST} = \frac{\sigma^2_{ST}(1 + F_{IS})}{2\bar{\sigma}^2_{IS} + \sigma^2_{ST}(1 + F_{IS})}. \tag{9}$$

It is clear that if each population is panmictic ($F_{IS} = 0$), the above expression reduces to

$$F_{ST} = \frac{\sigma^2_{ST}}{2\bar{\sigma}^2_{IS} + \sigma^2_{ST}}. \tag{10}$$

This equation is the one classically used by various authors (*e.g.*, see PODOLSKY and HOLTSFORD 1995). If instead, as is assumed in this paper, the species is mainly selfing ($F_{IS} = 1$), the expression of F_{ST} becomes

$$F_{ST} = \frac{\sigma^2_{ST}}{\bar{\sigma}^2_{IS} + \sigma^2_{ST}}, \tag{11}$$

which is the equation used in this paper.